



09/13/2023

2023.09.13 Project Skunkworks: Power Cloning™ T5-NotI Assemblyase™**Power Cloning™ T5-NotI Assemblyase™**

The first core competency of Radegen Biotechnology is cloning reagents. Our R&D has led to the development of cloning reagents that use in vitro homology directed repair for circularizing a cloning construct. The system is designed for use of any compatible plasmid or the included PpuOriC™ pFA plasmids. pFA plasmids contain the pUC high copy replication origin and are designed for propagation and maintenance in *E. coli* or *P. putida*. The PpuOriC™ feature of the pFA plasmid is for in vitro plasmid enrichment during a cloning reaction and does not interfere with plasmid function in *E. coli*.

T5-NotI Assemblyase™ - A bacterial cell free lysate reagent that is competent for homology directed repair of DNA. This system can either be produced from a RecA- strain of bacteria expressing the Lambda red operon or from a RecA+ bacterial strain. This system is specifically designed for assembling dsDNA fragments with 15-40 nt 3' overhangs produced from DNA excised from a donor plasmid. The Assemblyase system contains T5 exonuclease, NotI and the associated methyltransferase. A dsDNA donor fragment bound to a plasmid is released by NotI digest. The dsDNA fragment is then processed by XthA and mismatches are removed by exonuclease digest on the 3' strand. Simultaneously, 80 nt 3' overhangs are produced by T5 digest of the 5' strand. In pure enzyme preparations of this golden-gate-like technology, the Assemblyase is replaced with a proofreading polymerase and a DNA ligase. This reagent is designed for the assembly of a single or multiple NotI donor plasmids into any destination plasmid. Excised donor fragments should be designed to contain homology arms between 15-40 nt. XthA is a 3'-5' exonuclease that removes terminal mismatches up to 10nt. Assemblyase™ for BsaI, BsmBI and SapI is XthA deficient.

>T5 Exonuclease

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MSKSWGKFIEEEEAEMASRRNLMIVDGTNLGFRFKHNSKKPFASSYVSTIQSLAKSYSARTTIVLGDKGKSVFRLEHLPEYKGNRDEKYAQRTEEEKALDEQFFEYLKDA
FELCKTTFPTFTIRGVEADDMAYIVKLIGHLYDHVWLISDTGDDWTLTLDKVSRSFSTTRREYHLRDMYEHNNVDDVEQFISLKAIMGDLGDNIRGVEGIGAKRGYNIIR
EFGNVLDIIDQLPLPGKQKYIQNLNASEELLFRNLILVDLPTYCVDAIAAVGQVDLDKFTKDILEIAEQ*
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>NotI

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MRSSTSVEPEGANFIAEFFGHRVYPEVSTEAARNQATGTCPFLTAAKLVETSCVKAETSRGVCVVNTAVDNERYDWLVCNRLDPLFMSAASRKLFGYGPTPLQFIA
APTADQAVRDGIREWLDRGVHVYVYFQEKLGELSISKTDSSEFSFDWTLAEVESIYPVPKIKRYGVLEIQTMDFHGSYKHAVGAIDIALVEGIDFHGWLPTPAGRAAL
SKKMEGPNLSNVFKRTFYQMAYKFALSGHQRCAGTGFAIPQSVWKSRLHLANPTLIDNGDGTFSLGDRNRDSENAWIFVFELDPDTPASPRPLAPHLEIRVNVDTLIDLA
LRESPRALGSPGPVATFTDKVEARMLRFWPKTRRRRSTTPGGQRGLFDA*
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>NotI.M1

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MSGPRLHVGISVCGSLTGLWITRSRRTVPRVVIPYDACVTSLSYSSAIDDPTEVRQGDAYDLASGLDPQSIDLIITSPPYWGMRTYGHDSVDLDEWVAEGNHATDVP
PYEWYREHGGLLGMEPIPEWFISHLVEIFERLRPALKLGGSVWVNLGDTYFARWSSIRSDDGRQGLGDNPRTRRKTTPMGGYRQEQQLMLIPSRFAIAMQDKRWILRNDLIWH
KPNVAPRPEKDRRLRAHEHFFHVLRPKEGRAKYYDYDTSAVEEGTRDVTNVNRSGSDGHSATFPDILIRPRIESSSPVGGVLVDPFAGTGRALGVAAELGRSAIGFELSE
EFTQAATRNAEASANALRLL*
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